REMARKS

Amendments to the Abstract:

Please replace the abstract with the new abstract enclosed.

Claim amendments:

Claims 1, 3, 27, 28, 57, 58, 60 and 62 were amended to ensure the preamble is in accordance with parts (a), (b) and (c). The term "comprising or suspected of comprising" has been inserted in place of the term "possibly containing".

Claims 12 and 41was amended by inserting the terms "comprising a column"

Claim 62 was amended by inserting the method steps of claim 1.

Claims 1, 58, 60 and 62 were amended by inserting the term "wherein the sample is approximately 100 µl". Claim 28 was amended by inserting the term "wherein the sample is approximately 700 µl.

Claims 9, 37, 38 and 54 have been canceled.

Claims 64 and 65 have been added. Support can be found in paragraphs [0098] and [0113]. Applicant believes that no new matter has been added.

Amendments to the Abstract

The Examiner objected to the abstract because of the inclusion of the word "comprising".

Applicant has amended the abstract by replacing "comprise" and "comprising" with "include" and "including" Please delete the abstract on file and replace it with the new abstract enclosed.

Marked-up copy of abstract:

> **Methods, systems and kits for the simultaneous or sequential analysis of one or more hormones by mass spectrometry are disclosed. The methods require minimal sample size and minimal preparation time. The methods include ~~comprise~~ ionizing the hormones and analyzing the hormones by mass spectrometry. In addition, methods, systems and kits for the simultaneous or sequential analysis of thyroid hormones are disclosed including ~~comprising~~ ionization of the thyroid hormones in the negative mode using an electrospray source.**

New abstract:

Methods, systems and kits for the simultaneous or sequential analysis of one or more hormones by mass spectrometry are disclosed. The methods require minimal sample size and minimal preparation time. The methods include ionizing the hormones and analyzing the hormones by mass spectrometry. In addition, methods, systems and kits for the simultaneous or sequential analysis of thyroid hormones are disclosed including ionization of the thyroid hormones in the negative mode using an electrospray source.

Claim rejections under 35 USC §112

The Examiner objected to claims 1-63 as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

(1) The Examiner states that Claims 1, 28, 58 and 60 are indefinite because the preamble and part (a) of the claims are not commensurate in scope with one another.

Applicant has amended claims 1, 3, 27, 28, 57, 58, 60 and 62 to ensure the preamble is in accordance with parts (a), (b) and (c). The term "comprising or suspected of comprising" has been inserted in place of the term "possibly containing". Support is found in paragraphs [0043] and [0044].

(2) The Examiner states that claims 12 and 41 recite the limitation "column" but there is insufficient antecedent basis for this limitation in the claims.

Applicant has amended claims 12 and 41 by inserting the terms "comprising a column".

(3) The Examiner states that claims 62-63 provide use of a mass spectrometer but the claim does not set forth any steps involved in the method/process. The Examiner further states that a claim is indefinite where it merely recites a use without any active, positive steps delimiting how this use is actually practiced.

Applicant has amended claim 62 by inserting the steps of claim 1.

Claim rejections under 35 USC §103

Kissmeyer et al.

The Examiner states that claims 1, 3, 6, 9-10, 12-16, 18, 20, 23, 25, 27-28, 30-31, 34, 38-39, 41-45,48-49,53, 55, 57-59, and 62-63 are obvious with regard to Kissmeyer et al.

Kissmeyer et al. disclosed the analysis of vitamin D (EB1089) and its derivatives in human and pig serum using a 1.0 ml sample using a laborious method of protein extraction, followed by MF C18 SPE column purification and further LC-MS analysis. Kissmeyer et al. did not disclose the analysis of thyroid hormone. The Examiner states that it would have been obvious to one having ordinary skill in the art at the time the invention was made to perform a method of conducting mass spectrometry of steroids or vitamin D analogs that would also be performed on another compound such as thyroid hormones, since mass spectrometry is measuring mass ratios and independent of the compound being analyzed.

To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, a prior art reference (or references) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in Applicants' disclosure. In re Vaeck, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991).

Applicant submits that since Kissmeyer et al. did not teach the analysis of thyroid hormones all the claim limitations are not found in the cited reference and therefore the claims cannot be obvious in view of Kissmeyer et al. Further, there was no motivation or suggestion in Kissmeyer et al. to analyze thyroid hormones. Finally, there is no reasonable expectation of success found in Kissmeyer et al. because Kissmeyer et al. did not disclose or suggest the analysis of thyroid hormones.

Regarding the Examiner's statement that "it would have been obvious to one having ordinary skill in the art at the time the invention was made to perform a method of conducting mass spectrometry of steroids or vitamin D analogs that would also be performed on another compound such as thyroid hormones, since mass spectrometry is measuring mass ratios and independent of the compound being analyzed", Applicant

submits that the claims are not merely directed to the analysis of thyroid hormones by mass spectrometry, but also to the preparation of the sample containing or suspected of containing thyroid hormones (steps (a), (b) and (c) of claim 1). Steroid hormones are chemically distinct from thyroid hormones and a skilled worker would not assume that the preparation of steroid hormones would be similar to the preparation of thyroid hormones for analysis by mass spectrometry. Clearly, the preparation of the sample is important, and one must conduct experiments to determine the preparation steps that yield accurate and consistent results. Through careful experimentation, the Applicant surprisingly determined that the preparation steps for the analysis of thyroid hormones (present application) and of steroid hormones (co-pending US 10/823,691) are similar, but that different sample sizes are recommended (see claims 37 and 38). The Examiner has cited numerous references that disclose the analysis of various steroid hormones and thyroid hormones by mass spectrometry, but all references differ in the preparation of the sample. A skilled worker having knowledge of the prior art would come to the conclusion that preparation of the sample is dependent on the type of steroid or type of thyroid hormone being analyzed, and would not come to the conclusion that the preparation of steroid hormones would be similar to that of thyroid hormones.

Accordingly, the claims are not obvious in view of Kissmeyer et al. because (i) the claim limitations are not present in Kissmeyer et al. and therefore the test for obviousness has not been met, (ii) Kissmeyer et al. does not suggest that the steps involved in the preparation and analysis of vitamin D would be successful in the preparation and analysis of thyroid hormones and therefore there is no reasonable expectation of success, and (iii) a skilled worker having knowledge of the prior art could not assume that chemically distinct compounds, like thyroid hormones and steroid hormones, would require similar preparation steps for their analysis by mass spectrometry.

The prior art teaches the analysis of many different steroid and thyroids, and the preparation of the samples differ in the methods taught by the prior art. For example, Kissmeyer et al. discloses the analysis of vitamin D analogs from a 1.0 ml sample of human or pig serum. Kissmeyer discloses the following method of preparation:

> The serum is protein precipitated with two volumes of acetonitrile then centrifuged. The supernatant is mixed with one volume of water and loaded to an Isolute MFC SPE column that was previously preconditioned with heptane, 2-propanol, methanol and acetonitrile-water (33:67). The column was successively washed with acetonitrile-water (33:67), methanol-water (70:30) and heptane. The vitamin D analogs were eluted with heptane-2-propanol (93:7). The organic solvent was evaporated under a stream of N at 40°C using a TurboVap LV Evaporation. The residues were reconstituted in 200 ul of methanol-1 M ammonium acetate-water (500:2:500) before analysis by LS/MS/MS.

In contrast, Fredline et al. discloses the analysis of aldosterone (another steroid) from blood, using a preparation procedure as follows:

> A 2 ml sample from of patient was pipetted into 15 ml glass culture tubes to which 500 mM ammonium formate buffer (1 mL pH 7.1) was added. Samples of <2mL were diluted 1:1 with water. Analytes were extracted with dichloromethane/diethyl ether (60/40, v/v) containing 0.4 pg/mL IS (6mL). Each tube was capped and mixed by inversion for 20 minutes. The organic layer was transferred into a 10mL glass culture tube and evaporated to dryness under nitrogen at 35°C. The residue was reconstituted in 50uL of mobile phase.

The above two methods demonstrate that the prior art teaches very distinct methods of sample preparation for different steroids. A skilled worker having knowledge of the prior art would conclude that each steroid requires a particular preparation method, and would further conclude that thyroid hormones would require a different preparation method than steroid hormones.

Applicant requests that the objection be withdrawn.

Jonsson et al.

The Examiner objected to claims 1-3, 8-10, 12, 14-16, 18, 20, 23, 25, 27-28, 30-31,36, 38-39, 41, 43-45 citing Jonsson et al. Jonsson et al. discloses a method and system for the determination of cortisol in saliva.

Jonsson et al. disclosed the analysis of one steroid, cortisol, from saliva. Jonsson et al. did not disclose the analysis of thyroid hormones. For similar reasons outlined in the argument for Kissmeyer et al, the claims are not obvious in view of Jonsson et al. because (i) all the claim limitations are not present in Jonsson et al., (ii) there is no reasonable expectation of success in Jonsson et al., and (iii) a skilled worker having knowledge of the prior art could not assume that chemically distinct compounds, like thyroid hormones and steroid hormones, would require similar preparation steps for their analysis by mass spectrometry. In fact, the prior art teaches numerous preparation methods, all of which are different and this suggests that each distinct compound would require a different method of preparation.

Kao et al.

The Examiner objected to claims 1, 3-4, 6, 9, 12-16, 18, 20, 23, 24, 27, 28, 30-32, 34, 38, 41-45, 4, 49, 52, 57-59 and 62-6333 citing Kao et al. Kao et al. discloses a method and system for simultaneously analyzing at least three components of the adrenal pathway using LC-tandem mass spectrometry.

Kao et al. discloses a method to assess adrenal dysfunction. The adrenal glands are endocrine glands that sit on top of the kidneys and are chiefly responsible for regulating the stress response through the synthesis of corticosteroids and catecholamines, including cortisol and adrenaline. The adrenal glands do not synthesize nor regulate thyroid hormones. Kao et al. did not disclose the analysis of thyroid hormones. For similar reasons outlined in the argument for Kissmeyer et al, the claims are not obvious in view of Kao et al. because (i) all the claim limitations are not present in Kao et al., (ii) there is no reasonable expectation of success in Kao et al., and (iii) a skilled worker having knowledge of the prior art could not assume that chemically distinct compounds, like thyroid hormones and steroid hormones, would require similar preparation steps for their analysis by mass spectrometry. In fact, the prior art teaches numerous preparation methods, all of which are different and this suggests that each distinct compound would require a different method of preparation.

Fredline et al.

The Examiner objected to claims 1, 3-6, 9, 12, 14-16, 18, 21, 23, 25, 27-28, 30-34, 38, 41, 43-45, 48, 50, 53, 55, 57-59 and 62-63 stating that Fredline et al. discloses a method and system for the determination of aldosterone in samples of plasma or blood.

For similar reasons outlined in the argument for Kissmeyer et al, the claims are not obvious in view of Fredline et al. because (i) all the claim limitations are not present in Fredline et al., (ii) there is no reasonable expectation of success in Fredline et al., and (iii) a skilled worker having knowledge of the prior art could not assume that chemically distinct compounds, like thyroid hormones and steroid hormones, would require similar preparation steps for their analysis by mass spectrometry. In fact, the prior art teaches numerous preparation methods, all of which are different and this suggests that each distinct compound would require a different method of preparation.

Leinonen et al.

The Examiner objected to claims 1, 3, 7, 12-18, 20, 22, 24, 27-28, 30-31, 35, 38, 41-46, 48-49, 52, 54, 57-59 and 62-63 citing Leinonen et al. Leinonen et al. discloses a method and system for the determination of anabolic steroids is urine using LC-mass spectrometry.

Leinonen et al. teaches the analysis of three "free" anabolic steroids in urine (oxandrolone, hydroxyl-4-chlorohydro-methyltestosterone, hydroxystanozolol). The urine samples were extracted by a complex process using sodium hydrogencarbonate-potassium carbonate mixture and extracted with diethyl ether.

For similar reasons outlined in the argument for Kissmeyer et al, the claims are not obvious in view of Leinonen et al. because (i) all the claim limitations are not present in Leinonen et al., (ii) there is no reasonable expectation of success in Leinonen et al., and (iii) a skilled worker having knowledge of the prior art could not assume that chemically distinct compounds, like thyroid hormones and steroid hormones, would require similar preparation steps for their analysis by mass spectrometry. In fact, the prior art teaches

numerous preparation methods, all of which are different and this suggests that each distinct compound would require a different method of preparation.

Vogeser et al.

The Examiner objected to claims 1, 3, 6, 11-16, 18, 20, 22, 25, 27-28, 30-31, 34, 4-45, 48-49, 52, 55, 57-59 and 62-63 citing Vogeser et al. Vogeser et al. discloses a method and system for the determination of cortisol in serum.

Vogeser et al. disclose the analysis of one steroid (cortisol) is serum samples precipitated with methanol/zinc sulfate in a three step extraction process.

For similar reasons outlined in the argument for Kissmeyer et al., the claims are not obvious in view of Vogeser et al. because (i) all the claim limitations are not present in Vogeser et al., (ii) there is no reasonable expectation of success in Vogeser et al., and (iii) a skilled worker having knowledge of the prior art could not assume that chemically distinct compounds, like thyroid hormones and steroid hormones, would require similar preparation steps for their analysis by mass spectrometry. In fact, the prior art teaches numerous preparation methods, all of which are different and this suggests that each distinct compound would require a different method of preparation.

De Brabandere et al. and Kissmeyer et al.

The Examiner objected to claims 1-3, 6, 9, 22, 23, 24, 26-27, 28, 29, 30-31, 34, 37-39, 41-45, 47-49, 51, 53, 55, 56-59, and 62-63.

De Brabandere et al. discloses a reference method for the determination of thyroxine in serum using a complex sample preparation method including extraction via multiple centrifugation steps followed by evaporation under a stream of nitrogen. The preparation time is estimated at between one and two and a half hours. The reference method is meant to be used to harmonize routine methods. The first few lines of De Brabandere et al. state: "In clinical chemistry, reference methods are the key to an accuracy based harmonization of routine methods. They are applied to certification of reference materials for determination of target values in external and internal quality

control materials and to the evaluation of routine methods on patient samples." Accordingly, the method disclosed by De Brabandere et al. is not meant to be used in the routine testing of patient samples, and that explains why the sample preparation is complex and lengthy. Further, although De Brabandere et al. avoid stating how much serum they use, it appears to be somewhere between 0.6mL and 1.5 mL.

Applicant has amended claims 1, 58, 60 and 62 by adding the term "wherein the sample is approximately 100 µl". Support for this range is found in paragraphs [0080], [0097], [0101], [0111], [0115] and claims 9, 37 and 38 as filed. This is an important aspect of the invention because it allows the analysis of thyroid hormones from small children and infants. It does not appear that De Brabandere et al. disclose a method for the analysis of thyroid hormone from a small sample of biological fluids (i.e. approximately 100 uL). Kissmeyer discloses the analysis of steroid hormones using a 1 ml sample. Since neither reference discloses the use of a sample within the 100 µl range, the new claims cannot be obvious in view of De Brabandere et al. and Kissmeyer et al. because all the claim limitations are not present in the cited references as is required by the test for obviousness.

In a similar manner, Applicant has amended claim 28 by adding the term "wherein the sample is approximately 700 µl". Support for this range is found in paragraphs [0080], [0097], [0101], [0111], [0115] and claims 9, 37 and 38 as filed. Claim 28 is directed to the analysis of a sample comprising both thyroid and steroid hormones. Neither De Brabandere et al. nor Kissmeyer et al. disclose the analysis of both steroid and thyroid hormones from a 700µl sample.

Applicant submits that the objection has been overcome.

Thienpont et al. and Kissmeyer et al.

The Examiner objected to claims 1-3, 6, 9, 12-20, 22, 25-27, 28, 29, 30-31, 34, 37-39, 41-45, 47-49, 51, 53, 55, 56-59 and 62-63 stating they are obvious with regard to Thienpont et al. in view of Kissmeyer et al. Thienpont et al. disclose a method for the determination of triiodo L-thyronine in serum using isotope dilution-GC/MS and isotope

dilution LC/MS/MS. Thienpont et al. use a maximum sample of 3 ml of serum and require a complex preparation including evaporation to dryness under nitrogen. As stated above, Kissmeyer et al. use a 1 ml sample. Neither Thienpont et al. nor Kissmeyer et al. use a sample of approximately 100 or 700 µl. The small sample size is important because this allows the analysis of thyroid and steroid hormones from infants and small children. Accordingly, the new claims cannot be obvious in view of Thienpont et al. and Kissmeyer et al. because all the claim limitations have not been met. Applicant submits that the objection has been overcome.

De Brabandere et al., Thienpont et al., Kissmeyer et al., Jonsson et al., Kao et al., Fredline et al., Leinonen et al. or Vogeser et al.

The Examiner states that claims 60-61 are obvious in view of the above cited references because it would have been obvious to one having ordinary skill in the art to incorporate all of the needed reagents and instrumentation required to analyze steroid and thyroid hormones in a kit form.

Applicant respectfully submits that Kissmeyer et al., Jonsson et al., Kao et al., Fredline et al., Leinonen et al. and Vogeser et al. do not disclose the analysis of thyroid hormones or any kits for the analysis of thyroid hormones. Since the claim limitations are not present in any combination of Kissmeyer et al., Jonsson et al., Kao et al., Fredline et al., Leinonen et al. and Vogeser, these claims cannot be obvious in view of these references.

In addition, although De Brabandere et al. and Thienpont et al. disclose the analysis of thyroid hormones, they do not disclose any kits, nor do they disclose any motivation to make a kit. Again, since the claim limitations are not present in these references they do not compensate for the deficiency of the "steroid" references. Accordingly, claims 60-61 cannot be obvious in view of these references. Further, De Brabandere et al. and Thienpont et al. disclose different methods to analyze thyroid hormones than those disclosed and claimed in the present invention. Accordingly, *had* De Brabandere et al. or Thienpont et al. disclosed a kit (which they do not), their kit would be different from the kit of the present invention. In particular, their kit would require more reagents to

perform their complex de-proteination and extraction procedures. The benefit of the kit of the present invention is its simplicity. Applicant requests that the objection be withdrawn.

CONCLUSION

Applicant believes that it has fully responded to the Examiner's concerns, and that the claims are in condition for immediate allowance.

Please charge any deficiency or credit any overpayment in any fee required for this response, including any petition fee, to Deposit Account No. 502651.

In the event that any issues remain, the Examiner is invited to telephone the undersigned at (416) 865-7367 with any proposal to advance prosecution.

Yours very truly,

October 29, 2007

Date

MaryAnne Arnoldo
Registration No. 58,287
Torys LLP
Suite 3000
79 Wellington Street West
Box 270, TD Centre
Toronto, Ontario
M5K 1N2
Canada